PATENT
Attorney Docket No. 201895

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Falck-Pedersen

Group Art Unit: 1632

Examiner: R. Schnizer

Serial No. 08/653,114

Filed: May 24, 1996

For: ADENOVIRUS GENE EXPRESSION SYSTEM

DECLARATION UNDER 37 C.F.R. § 1.132

I, Imre Kovesdi, hereby declare that:

1. I have more than fifteen years of experience in adenovirus research. The goal of my research has been, and continues to be, advancing gene therapy by providing novel and/or improved adenoviral vector systems (i.e., adenoviral vectors and the complementary cells in which the adenoviral vectors can be propagated).

2. Currently, I am Vice President and Chief Scientific Officer at GenVec, Inc. (which is a licensee of the subject patent application). Prior to this position, I was Vice President of Discovery Research, and directed all of my company's adenovirus related research, development and manufacturing. I was the first scientist hired by GenVec.

3. Formerly, as leader of the Eukaryotic Gene Expression Group at Lederle Laboratories, I effected the design and construction of recombinant adenovirus systems for the study of transcription factors and the development of rapid screens to identify agents targeted against viral transactivators.

4. My work with adenoviruses began with my postdoctoral appointment at the Rockefeller University, where I investigated gene regulation in adenoviruses.

My Ph.D. in Molecular Biology from Simon Frasier University provided expertise in manipulating complex genes and cell systems, such as those inherent to adenoviral research. Along these lines, my experience also includes the cloning and characterization of two novel human genes encoding heparin-binding proteins that stimulate neurite outgrowth from perinatal neurons and the identification of the E2F transcription factor, which is key in cell cycle regulation.

5. I am the author or co-author of over eighty research papers. I am a co-inventor of several patents and patent applications and am a member of numerous scientific organizations relevant to my research. I also have been a recipient of academic honors for my scientific research. This is all set forth in the attached Curriculum Vitae.

6. I have reviewed the specification of the above-identified patent application. I also have read the Office Action dated August 23, 1999, and understand that claims 1-4, 13-15 and 17 have been rejected as allegedly being obvious in view of Kirshenbaum et al., *J. Clin. Invest.*, *92*, 381-389 (1993); Quantin et al., *Proc. Natl. Acad. Sci. USA*, *89*, 2581-2584 (1992); or Stratford-Perricaudet et al., *J. Clin. Invest.*, *90*, 626-630 (1992); in view of Huang et al., *Nucl. Acid Res.*, *18*(4), 937-347 (1990); Choi et al., *Mol. Cell. Biol.*, *11*(6), 3070-3074 (1991); Keating et al., *Exp. Hematol.*, *18*, 99-102 (1990); and WO 91/00747 (KabiGen et al.).

7. On the basis of my extensive experience with adenoviral vectors, I do not believe that an ordinary researcher in about December, 1993, reasonably would have believed that incorporation of a regulatory element, in particular, a splice site, which is characterized as being able to enhance expression in other types of DNA transfer vectors, would function in adenovirus to similarly enhance expression.

8. The nature of adenoviral vectors is a great deal more complex than other types of expression systems, such as plasmids. Adenoviruses are non-enveloped viruses containing a linear, double-stranded DNA genome. The adenoviral genome is

larger and its organization is more complex than other DNA and viral vectors, such as retrovirus and adeno-associated virus. For example, each of the E1-E4 transcription units is operably linked to separate promoters and encodes several alternatively spliced mRNAs. Adenoviruses infect cells using specific cellular receptors and, as such, require the necessary genetic elements and appropriate cellular conditions to produce coat proteins and encapsidate into viral particles. Adenoviral vectors require the interaction of numerous *cis* and *trans*-acting factors, both adenoviral and cellular, for successful infection and expression of proteins to occur. Many of such factors are not required for transfection of plasmid DNA into a cell. In addition, within a host cell, the adenoviral genome is non-covalently bound to the nuclear matrix through the adenoviral terminal protein. A plasmid typically exists as an episomal, free-floating DNA template.

9. Due to the complex nature of adenovirus, regulatory sequences can have different activity in adenovirus than in other expression systems. For example, the relative strength of a promoter in an adenovirus is not identical to the activity of the same promoter in a different type of vector. For example, the SV40 early or late promoters are known to be strong promoters in nature. However, SV40 promoters are relatively weak promoters when used to direct transcription in adenovirus (Prevec, *Acquired Immune Deficiency Syndrome*, *4*, 568-576 (1991)). Furthermore, the specificity of a promoter can differ when used in adenovirus. For instance, mlc, a promoter specific for heart tissue, does not retain its tissue-specificity when used in an adenoviral vector (Shi, *Human Gene Therapy*, *8*, 403-410 (1997)).

10. With respect to splicing, unlike other expression systems, adenoviral genomes comprise numerous splice sites. Indeed, every adenoviral gene, with the exception of the IVa2 gene, is spliced, and some genes are spliced extensively. In fact, over 30 species of mRNA corresponding to the early regions of adenovirus have been identified (Berkner, *BioTechniques*, *6*(7), 616-624 (1988)). Indeed, mRNA splicing and the use of overlapping reading frames allows the E1 region to encode many proteins. The identification and preliminary characterization of proteins

encoded by the E1 region alone took nearly a decade of research. The adenovirus major late transcription unit can be processed into at least 20 mRNAs. The extensive splicing of adenoviral transcripts hinders the predictability of success when using heterologous splice sites in adenoviral vectors.

11. Moreover, splicing in adenovirus is a highly regulated process and alternative splicing is common. In many adenoviral genes, splicing varies during the course of infection. For example, the mRNAs resulting from the major late transcription unit (MLTU) have a common 5' splice site, which can be spliced to more than 15 different 3' splice sites. Splicing of the MLTU changes depending on where the virus is in the course of infection. Alternative splicing of the MLTU differs from splicing events in other organisms in that an "on-off switch" associated with 3' splicing elements is not involved. Instead, alternative splicing of the MLTU is a result of activation of additional 3' splice elements, which leads to an increased number of mRNA species (see, for example, Muhlemann et al., *J. Virol.*, *69*(11), 7324-7327 (1995)). Alternatively, the E1a transcript is spliced into three different mRNA species, the 13S, 12S, and 9S. In the case of E1a, each mRNA species utilizes different 5' splice sites while sharing a common 3' splice site. Splicing from the 5' splice sites of the E1a region has been shown to be dependent on both *cis*- and *trans*-acting factors. Thus, due to the tightly controlled splicing mechanism, it is not possible to reasonably predict the activity of a heterologous splice site in an adenoviral vector.

12. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date: May 30, 2000

Imre Kovesdi, Ph.D.